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FACTORS AFFECTING THE B-VITAMIN
CONTENT OF COTTAGE CHEESE

By
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A THESIS

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science
Department of Dairy Science

Under the Supervision of Dr. Khem M. Shahani

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INTRODUCTION

Cottage cheese is nutritionally an excellent food. It serves as an economical, low-calorie source of a high quality protein. Cottage cheese is also a good source of minerals and vitamins.

The production and consumption of Cottage cheese is continuously increasing. There were 787,410,000 pounds of creamed Cottage cheese produced in the United States in 1963 (63), and its per capita consumption in the United States has increased from 2.6 pounds in 1950 to 4.8 pounds in 1960 (42).

While the B-vitamins have been shown to be essential for the human health, the dietary requirements of only some of the B-vitamins have been established. This has been due to a lack of knowledge concerning the nutritive requirements of individuals and the vitamins provided by the diet.

Sullivan et al. (58) reported that dairy products play an important role in balancing the diet, particularly with respect to vitamins.

The importance of dairy products as a source of vitamin B₁₂ in the human diet was observed by Wokes et al. (66). These workers reported that vegetarians whose diet included dairy products showed no visible illnesses. However, another group of vegetarians who eliminated dairy foods from their diet, developed definite illnesses which were apparently due to a deficiency of vitamin B₁₂ since the symptoms were alleviated by administering vitamin B₁₂.

Although B-vitamin values found in cow's milk are quite numerous, the literature contains relatively limited information on the vitamin

content, particularly the B-vitamins like niacin, B₆, B₁₂ and folic acid content of Cottage cheese. Even fewer figures are available for B-vitamins in the whey produced in the manufacture of Cottage cheese. More information is needed concerning the B-vitamin contents of dairy foods in order to help appraise the nutritive value of diets containing these foods and to assist in estimating approximate human requirements.

Since Cottage cheese is an important food source and constitutes a major dairy product, the factors which influence the nutritional quality of Cottage cheese cannot be overlooked. The purpose of this study was to investigate possible factors which may influence the B-vitamin content of Cottage cheese.

LITERATURE REVIEW

The work which has been done relative to the vitamins in cheese was mainly concerned with the vitamins A, D, B₁, B₂ and ascorbic acid. Little attention has been given to the other vitamins of the B-complex group, like niacin, B₆, B₁₂ and folic acid in Cottage cheese. The significance of these B-vitamins is very important as accessory growth factors in human nutrition and in the metabolism of carbohydrates, proteins and fats.

Niacin is a very important vitamin in human nutrition. Its deficiency in humans is characterized by such symptoms as symmetrical dermatitis, diarrhea, enteritis and a degeneration of motor and sensory nerves. This vitamin serves as part of coenzymes I, II and III which aid in transfer of hydrogen in the oxidation of breakdown products of proteins, fats and carbohydrates (51).

Vitamin B₆ functions as a coenzyme in the synthesis of unsaturated fatty acids, in the synthesis and breakdown of amino acids, and in decarboxylation and transamination reactions. Deficiencies of vitamin B₆ in the human diet result in the following symptoms: conjunctivitis, epileptiform convulsions in infants, sensory neuritis, anorexia, lethargy and confusion (51).

Vitamin B₁₂ plays an important role in human nutrition in that it is essential for the normal development of red blood cells, for the biosynthesis of nucleic acids, aids in the utilization of methyl groups, and acts as a growth factor for children. A deficiency of vitamin B₁₂ in man can result in such diseases as pernicious and nutritional anemia

and sprue (51).

Folic acid in man is necessary for normal growth, stimulates reticulocytosis, is essential for normal metabolism of growing cells and tissues and is necessary for the synthesis of nucleic acids. Deficiency symptoms of folic acid in humans are nutritional cytopenia and leucopenia (51).

Although considerable research has been carried out concerning factors influencing the vitamin content of several types of cheese (7,8,13,16,21,55,58,60), there is little information available in the literature concerning the factors affecting the B-vitamin content of Cottage cheese.

With the information presently available in the literature, it is apparent that the levels of B-vitamins in cow's milk, the effect of feeding, season and stage of lactation on the vitamin content is fairly well established. The interest at present seems to be centered on how the different production and processing operations of dairy products affect the vitamin content and the nutritive quality of the product.

The B-complex vitamins are generally assayed by microbiological methods, because microbiological methods offer a higher degree of sensitivity, specificity and consistency than biological or chemical methods. The lactic acid bacteria have been found to be especially useful for the assay of B-vitamins because of their need for these growth factors.

Niacin. The most common procedure for the estimation of niacin is the microbiological method, using Lactobacillus plantarum as the assay organism. A summary of the niacin content of milk, whey and

Cottage cheese, as reported in the literature, is presented in Table I. As can be seen from the table, the average niacin content of whole milk was reported to range from 46 to 91 ug per 100 ml of milk. The average niacin content of dried skimmilk ranged from 1200 to 1800 ug per 100 g.

The average niacin content of Cottage cheese varied between 70 and 120 ug per 100 g. Only one value has been reported for the niacin content of dried whey, 1200 ug per 100 g (7).

Vitamin B₆. Vitamin B₆ is commonly assayed microbiologically, either by using the mold mutant, Neurospora sitophila, or by the yeast, Saccharomyces carlsbergensis.

Hodson (30) reported that in comparing the N. sitophila with the S. carlsbergensis method of vitamin assay, the two methods gave similar results when applied to fresh or pasteurized milk or to nonfat dry milk solids. However, when applied to evaporated milk, the N. sitophila method gave higher results than the S. carlsbergensis assay.

The vitamin B₆ contents of milk and Cottage cheese have been compiled and are presented in Table I. The average vitamin B₆ content of cow's milk was found to vary from 20 to 67 ug per 100 ml. The average vitamin B₆ content of reconstituted evaporated milk (73 ug per 100 g) was found to be slightly higher than the reported range for regular cow's milk. The average content of vitamin B₆ found in skimmilk ranged from 51 to 66 ug per 100 ml.

The average vitamin B₆ content of Cottage cheese ranged from 44 ug per 100 g for plain curd to 56 ug per 100 g for creamed curd.

TABLE I

NIACIN, B₆, B₁₂, AND FOLIC ACID CONTENT OF MILK AND MILK PRODUCTS AS REPORTED IN THE LITERATURE

Product	Niacin	B ₆	B ₁₂	Folic acid
	ug/100 ml or ug/100 g			
Milk ^a	46 (55) 61 (35) 67 (20) 76 (2) 80 (57) 91 (28)	20 (32) 33, 35 (30) 65 ^e (27) 67 (27) 73 ^d (27)	0.30 (17) 0.39 (50) 0.40 (12) 0.41 (43) 0.63 (64) 0.71 (23)	0.26 (10) 0.30 (40) 0.57 (60) 0.60 (62)
Skimmilk ^a	1200 ^c (7) 1800 ^c (46)	51.5 ^f (24) 54 (24) 66 ^f (27)	0.38 (26) 0.43 (11) 0.47 (12)	0.7, 0.9 (39)
Whey ^b	1200 ⁱ (7)		2.0, 2.5 ⁱ (38) 2.2 ⁱ (23)	3 ⁱ (67) 90 ⁱ (37)
Cottage cheese ^b	70 (2) 112 ^g (56) 120 ^h (56)	44 ^g (25) 53 ^g (56) 56 ^h (56)	0.8, 0.88 (22)	23.6 ^h (56) 23.7, 46.5 (62) 42.9 ^h (56)

() Reference note - When the same author has more than one figure for the same product, different assay methods were used.

^aMost figures for milk and skimmilk are in ug/100 ml.

^bMost figures for whey and Cottage cheese are in ug/100 g.

^cDried skimmilk.

^dReconstituted evaporated milk.

^eReconstituted dried whole milk.

^fReconstituted dried skimmilk.

^gPlain curd.

^hCreamed curd.

ⁱDried whey.

Vitamin B₁₂. Both biological and microbiological methods are used for the assay of vitamin B₁₂. The biological procedures include the rat and chick assay. The microbiological assay of vitamin B₁₂ employs several strains of Lactobacillus leichmannii; a mutant strain of Escherichia coli; an algal flagellate, Euglena gracilis; or a protozoa, Ochromonas malhamensis.

The average values for the vitamin B₁₂ potency of milk (22) and for most types of cheese (23), using the rat assay were reported to be higher than those obtained by the microbiological methods. However, Peeler et al. (52) observed that the chick growth and microbiological methods gave comparable results.

The determination of vitamin B₁₂ in cow's milk, using L. leichmannii, E. coli, E. gracilis and O. malhamensis have been reported to give similar results (19,43,50).

A review of the values reported for the average vitamin B₁₂ concentrations in milk, whey and Cottage cheese is presented in Table I. A large number of vitamin B₁₂ values have been reported for cow's milk, and the values range from 0.30 to 0.71 ug per 100 ml. The vitamin B₁₂ content of skim milk ranged from 0.38 to 0.47 ug per 100 ml, Cottage cheese from 0.8 to 0.88 ug per 100 g, and dried whey ranged from 2.0 to 2.5 ug per 100 g.

Folic Acid. Methods commonly used for the assay of folic acid include the biological chick assay and the microbiological methods, using either Streptococcus faecalis or Lactobacillus casei as the test organism. The use of these two organisms for the assay of folic acid

gave good results which were quite reproducible (59). Hodson (29) reported that the chick method of assay for folic acid appeared to give higher results than those obtained by either L. casei or S. faecalis.

A review of the literature concerning average folic acid contents found in milk, whey and Cottage cheese is presented in Table I. The average content of folic acid in cow's milk reportedly ranges from 0.26 to 0.60 ug per 100 ml. Only two values have been reported for the folic acid content of skimmilk--0.7 and 0.9 ug per 100 ml.

As reported in the literature, the concentration of folic acid in Cottage cheese ranged from 23.6 to 46.5 ug per 100 g, in fluid whey 3 ug per 100 g and in dried whey 90 ug per 100 g.

Synthesis of B-complex Vitamins by Cheese Starter Organisms. A considerable amount of work has been done concerning the B-vitamin requirements of lactic organisms; however, little is known regarding the synthesis of B-vitamins by such organisms. An examination of the growth factor requirements and assays of growth media of lactic acid cultures used in the manufacture of Cottage cheese may indicate whether certain B-vitamins are synthesized or utilized during the cheese making process. A more highly nutritional Cottage cheese than normally manufactured could possibly be made by the selection of commercial starter cultures that are high producers of B-complex vitamins.

Niacin. Several workers (1,45,47,54) studied the nutritional requirements of several strains of S. lactis, S. cremoris and other starter organisms and reported that all strains studied required niacin

for growth. However, Tevilevich (61) isolated several strains of S. lactis from cream and other sources that were capable of synthesizing niacin.

Vitamin B₆. In a nutritional study of 36 strains of S. lactis, Niven (45) observed that although the cultures appeared to possess a limited ability to synthesize pyridoxine, the addition of pyridoxine to the growth medium stimulated the growth of all strains. This stimulatory and nonessential nature of vitamin B₆ was also observed for practically all of a total of 57 strains of S. lactis and S. cremoris (1,54).

Vitamin B₁₂. Laganovskii (33) investigated the means for raising the vitamin B₁₂ activity of milk and observed that souring the milk with homofermentative lactic acid bacteria had no effect upon its vitamin B₁₂ content. He (34) also studied the synthesis of B₁₂ by 27 strains of lactic acid organisms and found that none of the homofermentative strains synthesized vitamin B₁₂ under any conditions. However, the heterofermentative strains tripled the content of B₁₂ when grown in milk.

Folic Acid. Studies of the nutritional requirements of a total of 102 strains of S. lactis and S. cremoris starter organisms revealed that folic acid was not required for growth (1,45,48,54).

Nurmikko (49) reported that during growth S. lactis and S. cremoris secreted folinic acid, an active derivative of folic acid, into the medium. The addition of phenylalanine to the medium stimulated growth slightly and increased the folinic acid production three-fold.

Nambudripad et al. (47) investigated the synthesis of growth factors by lactic acid bacteria in dahi (a fermented milk in India), and reported that S. lactis, S. thermophilus, L. bulgaricus, L. helveticus and L. acidophilus increased the folic acid content. The same authors reported that S. lactis and S. thermophilus also increased the folinic acid content of dahi.

Effect of Rennet. Rennet is used in the making of Cheddar cheese to produce a particular type of a firm, elastic curd. In the manufacture of Cottage cheese, employing rennet, the amount of rennet used is only about one-hundredth of the amount used for Cheddar cheese, and the purpose is not to coagulate the milk but to help prevent shattering and matting of the curd particles.

The use of rennet in the making of Cottage cheese has been found to increase the production of desirable curd characteristics. Cottage cheese, manufactured by the combined action of rennet and acid development, produces a low-acid, sweet-curd type of Cottage cheese. Phillips (53) observed that rennet was particularly effective for proper curd formation when flash pasteurization was used for the skimmilk. He reported that the amount of rennet needed usually ranged from 0.5 to 1.5 cc per 1000¹ lb of skimmilk. When rennet was not added to the milk, excessive shattering of the curd occurred during the cooking process.

¹To conform with the dairy industry literature, units of weights and measures are given in the U.S. System. In the section on "Experimental Methods", however, the units at several places are cited in the metric system in order to be consistent with the original reference articles.

Gould and Thurston (18) conducted experiments in which they varied the amount of rennet used and the whey acidity at the time of cutting. Batches of Cottage cheese were made using rennet extract at the rate of 0 to 3.3 ml per 1000 lb of skimmilk while varying the whey acidities at the time of cutting. They found that the most desirable curd characteristics were obtained when coagulation occurred just prior to the time the right acidity for cutting the coagulum was reached. When cutting the coagulum at a whey acidity of 0.51 to 0.55, they recommended that fresh rennet be used at the rate of 1.25 cc per 1000 lb of skimmilk. Lower amounts of rennet resulted in excessive matting, whereas higher amounts caused excessive shattering during the early stages of cooking. The authors concluded that the coagulating function of rennet was secondary in importance to its action of preventing curd matting during cooking. They recommended that the amount of rennet should be slightly less than that which causes disintegration of the curd during the Cottage cheese making operation.

Van Slyke and Price (65) reported that when Cottage cheese was made without rennet, the yield from identical skimmilk was usually slightly less than if rennet was used in the cheese making. They obtained yields of 13 to 14.5 lb of Cottage cheese curd per 100 lb of skimmilk when the curd was made with rennet. They also reported yields of up to 18 lb of rennet-type Cottage cheese from 100 lb of skimmilk without exceeding the limit of 80% moisture.

Cheese made by the addition of rennet to the milk has been shown to contain more calcium bound in the curd than if the cheese was made by

acid coagulation (5,41). Since nicotinic acid and folic acid are acidic in nature and therefore, may exist as salts of calcium, it was thought that an increase in the calcium content, due to the addition of rennet, might produce a corresponding increase in the vitamin content of Cottage cheese.

Effect of Calcium Chloride. Calcium chloride may be added to skimmilk used in the manufacture of Cottage cheese. The purpose of adding calcium chloride is to improve the coagulating properties of the skimmilk and to aid in the firming of the curd during heating. The amount of anhydrous, food-grade calcium chloride is limited by Federal definitions (15) and is not to exceed 0.02% of the weight of the skimmilk.

Phillips (53) indicated that the purpose of adding calcium chloride was to remedy deficiencies in the milk. He also reported that a small amount of calcium chloride would aid in obtaining proper coagulation of skimmilk that was flash pasteurized.

Dill et al. (14) reported that the addition of calcium chloride to pasteurized skimmilk used in the manufacture of Cottage cheese affected only slightly the yield of curd. However, the addition of calcium chloride was especially desirable when the heat treatment or solids-non-fat content of the milk were increased.

Van Slyke and Price (65) recommended the use of calcium chloride when a deficiency of calcium in the milk supply was suspected. They found that it was beneficial to use calcium chloride when excessive heat treatment in pasteurizing the skimmilk tended to interfere with the

normal functioning of rennet.

Since preliminary trials with rennet addition to the milk in cheese making indicated that a relationship between calcium and the acidic vitamins may exist, it was thought that the addition of calcium chloride to the Cottage cheese making process may affect the calcium, and consequently the niacin and folic acid content also.

SCOPE OF INVESTIGATION

There has been very little work done relative to the amounts of B-vitamins found in Cottage cheese or to the factors affecting its vitamin content.

Therefore, the purpose of this study was to investigate the vitamin content of Cottage cheese and to study the factors which may alter the content of certain B-vitamins. The specific objectives were as follows:

1. To determine the niacin, B₆, B₁₂ and folic acid content of skimmilk and Cottage cheese and the retention of these vitamins in the curd from the milk.
2. To study the synthesis of niacin, B₆, B₁₂ and folic acid during the 16-hr setting period used in making Cottage cheese, and to determine whether the vitamin synthesis by the starter was partly responsible for the vitamin content of the finished cheese.
3. To determine the effect of storage of Cottage cheese up to two weeks upon its niacin, B₆, B₁₂ and folic acid contents.
4. To investigate the addition of rennet to milk used in the manufacture of Cottage cheese upon the calcium, nicotinic acid and folic acid content in order to establish whether there existed any relationship between the calcium and the vitamin contents.
5. To investigate the addition of calcium chloride to milk used in the making of Cottage cheese and to determine

whether a relationship existed between the added calcium salt and the nicotinic acid and folic acid contents.

EXPERIMENTAL METHODS

The first phase of the study was to determine the vitamin content of milk, whey and Cottage cheese and to determine the effect of the starter and of the storage upon niacin, B₆, B₁₂ and folic acid content of Cottage cheese.

Vitamin Content of Milk, Whey and Cottage Cheese. Three separate batches of Cottage cheese were manufactured in the University dairy plant, using the long-set method. Two hundred gallon batches of fresh skim milk pasteurized at 145 F for 30 min were used for making each batch of the cheese. The starter used consisted of a commercial cheese culture maintained at the University dairy plant. Vitamin analyses were made for niacin, B₆, B₁₂ and folic acid on the three separate batches of skim milk and on the fresh dry curd and whey which was produced. Based on the average yield for the three trials of cheese, 5.78 lb of skim milk produced 1 lb of Cottage cheese curd. Then, on the basis of vitamin content of the skim milk used, the per cent retention of vitamin in the curd from the milk was calculated as follows:

$$\text{Per cent retention} = \frac{\text{Vitamin in curd} \times 100}{\text{Vitamin in milk} \times 5.78}$$

Vitamin Assay Procedures. The methods of vitamin analyses used in this investigation consisted of slight modifications of those described by Gregory et al. (19,20), Lichtenstein et al. (36) and Shahani et al. (56). The vitamin contents of milk, whey or cheese curd were determined immediately while the products were fresh, or the samples

were held frozen at -15 F until the assay could be made. The methods of vitamin analysis are described below.

Niacin. Thirty grams of Cottage cheese were blended with 90 ml of distilled water. To 40 g of this mixture, 100 ml of 1.0 N sulfuric acid was added and autoclaved at 15 psi for 30 min, cooled, brought to pH 6.8 with 20% sodium hydroxide, diluted to 200 ml with glass distilled water and filtered.

For the determination of niacin in milk or whey, a 20 g sample was mixed with 100 ml of 0.2 N sulfuric acid, autoclaved at 10 psi for 30 min, cooled and filtered.

The niacin content of the filtrates was then determined microbiologically, using L. plantarum ATCC 8014 as the assay organism. A standard curve ranging from 0 to 0.5000 ug niacin per tube was prepared along with the cheese, milk and whey extracts. The contents of the tubes were brought to 5 ml with glass distilled water, 5 ml of basal medium was added; the tubes were capped with stainless-steel covers, autoclaved for 10 min at 15 psi, and cooled. Each tube, except for the control tubes, was aseptically inoculated with two drops of a 16-hr suspension of L. plantarum which was washed twice with sterile isotonic salt solution and resuspended to a turbidity of 79-83% transmission at 640 mu. The tubes were then incubated for 18 hr at 37 C, titrated to an end-point of pH 6.8 with bromthymol blue, and calculations made for niacin content.

Vitamin B₆. Ten grams of Cottage cheese were blended with 95 ml

of 0.05 N hydrochloric acid. Eight milliliters of this blended mixture were mixed with 86 ml of 0.05 N hydrochloric acid and autoclaved at 15 psi for 4 hr, cooled, adjusted to pH 4.5 with 5.0 N sodium hydroxide, diluted to 100 ml with distilled water and filtered.

For the assay of B₆ in milk or whey, a 10 g sample was mixed with 900 ml of 0.05 N sulfuric acid, autoclaved at 15 psi for 5 hr, cooled, diluted to 1000 ml, adjusted to pH 4.6 and filtered.

The vitamin B₆ content of the filtrates was then assayed using S. carlsbergensis ATCC 9080 as the test organism. All dilutions for the standard curve and extracts were prepared in duplicate 50 ml Erlenmeyer flasks. The contents of the flasks were all adjusted to a volume of 5 ml with distilled water, 5 ml of basal medium added, the flasks plugged with cotton, autoclaved 10 min at 15 psi and cooled. Each flask, except the control, was aseptically inoculated with one drop of a 16-hr suspension of S. carlsbergensis which was washed with sterile isotonic salt solution and standardized to 73-76% transmission at 640 mu. The flasks were then incubated for 16-18 hr at 30 C with shaking, the turbidity of each flask was read spectrophotometrically at 640 mu on a Coleman Model 11 Spectrophotometer, and calculations were made for the B₆ content of the samples.

Cyanocobalamin or B₁₂. Twenty grams of Cottage cheese were mixed with 10 ml of buffer-bisulfite extractant, diluted to 200 ml with glass distilled water, blended, autoclaved at 15 psi for 10 min and cooled. Ten milliliters of this mixture were then diluted to 100 ml, and 10 ml of this dilution were diluted to 50 ml with distilled water and filtered.

For the preparation of milk or whey for B₁₂ assay, a 10 g sample was mixed with 10 ml of distilled water, the pH adjusted to 4.6 with 0.1 N hydrochloric acid, 10 drops of a 1% sodium cyanide solution added and the volume made up to 90 ml with distilled water. After autoclaving at 10 psi for 10 min, the sample was cooled, the pH adjusted to 5.5 with 0.1 N sodium hydroxide, the volume was adjusted to 100 ml and filtered.

The vitamin B₁₂ content of the filtrates was then determined using L. leichmannii ATCC 7830 as the assay organism. The experimental and standard tubes were autoclaved and inoculated with one drop of a 16-hr inoculum of L. leichmannii which was centrifuged and serially diluted to a concentration of 0.0001 to 0.11 mg dry cells per 10 ml of micro inoculum broth. After incubation for 16 hr at 37 C, the results were determined spectrophotometrically at 620 mu and the B₁₂ content of the samples calculated.

Folic Acid. Ten grams of Cottage cheese were blended with 100 ml of 0.2 M phosphate buffer, 5.5 ml of the blended mixture placed in a flask and heated 5 min at 100 C. The sample was then cooled, mixed with 20 mg of acetone-extracted chicken pancreas enzyme and incubated at 37 C for 24 hr. Following the incubation, the sample was placed in a boiling water-bath for 5 min, cooled, diluted to 100 ml, extracted with ethyl ether and filtered. Sixty milliliters of this filtrate were then diluted to 100 ml for the assay.

For the preparation of milk or whey extracts for the folic acid estimation, a 10 g sample was mixed with 10 ml of 0.2 M phosphate buffer, diluted to 100 ml, autoclaved for 15 min at 10 psi, adjusted to pH 4.6

and filtered.

The folic acid content of the filtrates was then determined, using S. faecalis ATCC 8043 as the assay organism. The standard and extract tubes were inoculated with 1 drop of an 18-hr culture which was washed and resuspended in solution to a turbidity of 73-76% transmission at 640 mu. After incubation of the tubes for 18 hr at 37 C, the results were read spectrophotometrically at 640 mu and the folic acid content of the samples calculated.

Effect of the Starter Upon the Vitamin Content of Cheese. Since preliminary trials showed that an unusually high per cent retention of folic acid occurred in the curd from the milk and a high concentration of vitamin occurred in the whey, it was felt that the Cottage cheese starter synthesized vitamins during the setting period.

Therefore, the rate of biosynthesis of niacin, B₆, B₁₂ and folic acid was determined during the 16-hr setting period used in the manufacture of Cottage cheese. To simulate the conditions prevailing in the cheese vat, pasteurized skimmilk was set up in 600 ml beakers using the normal long-set method of making Cottage cheese, and the vitamin assays were run at 4-hr intervals during the 16-hr coagulation period.

Then, on the basis of the vitamin content of the 16-hr coagulum, the per cent retention values of the four vitamins were calculated to take in account the synthesis of vitamins during the setting period as follows:

$$\text{Per cent retention} = \frac{\text{Vitamin in curd} \times 100}{\text{Vitamin in 16-hr coagulum} \times 5.78}$$

Effect of Storage Upon the Vitamin Content of Cheese. For this study, samples of Cottage cheese were obtained from the batches of cheese made in the first phase of study—the vitamin content of milk, whey and Cottage cheese. The fresh dry curd was creamed using a ratio of 66 parts of curd to 26 parts of 18% fat cream and stored in a refrigerator at 50 F for two weeks. Vitamin assays were determined for niacin, B₆, B₁₂ and folic acid on the dry curd, fresh creamed curd and after 1 and 2 weeks.

Effect of the Addition of Rennet to Milk Upon the Calcium and Vitamin Content of Cheese. Cottage cheese preparation is primarily an acid coagulation process, as compared to the rennet coagulation character of Cheddar cheese. The effect of rennet has been shown to increase the calcium content of rennet-curd about 7 to 10 times over the amount of calcium found in an acid-type curd. Niacin and folic acid, being acidic in nature, may exist as salts of calcium in cheese. Therefore, in order to study the possible interrelationship of calcium and the vitamin content of Cottage cheese, studies were made to determine the effect of the addition of different quantities of rennet to milk during the manufacture of Cottage cheese upon the calcium, niacin and folic acid content.

In this study, the short-set method of Cottage cheese manufacture was used. This method made it possible to prepare four batches of cheese during the same day. Four separate trials consisting of 16 batches of cheese were made in this phase of study. For the preparation of the cheese curd, rennet was added in concentrations of 1, 2 or 3 oz per 1000 lb of milk. A standard technique was employed for making the cheese,

which consisted of inoculating the milk with 6% starter at 90 F, incorporation of the rennet at 0.30% acidity and cutting the curd when the whey acidity reached 0.30%. The acidity was allowed to develop for 30 min after which the temperature was brought to 120 F; the whey was drained and the curd was washed with cold water.

Determinations were then made for calcium, niacin and folic acid in the skimmilk, whey and cheese curd. Vitamin assays were made as described earlier and the calcium content was determined chemically by the standard oxalate precipitation and potassium permanganate titration method outlined by the Association of Official Agricultural Chemists (3) for microquantities of calcium. A 2 g sample of milk, whey or cheese curd was weighed into a crucible, ignited in a muffle furnace at 500 C, dissolved in hydrochloric acid, diluted to 100 ml and filtered. Fifteen milliliters of the filtrate were placed in a conical-tip centrifuge tube with 2 ml of saturated ammonium oxalate. Using methyl red as the indicator, the pH of the solution was adjusted to 5.0. After standing for 3 hr, the precipitate was centrifuged and washed three times. After the last supernatant had been removed, the precipitate was dissolved in dilute sulfuric acid and titrated with 0.02 N potassium permanganate at 80-90 C.

Effect of Calcium Chloride Addition to the Milk Upon the Calcium and Vitamin Content of Cheese. Since preliminary trials with rennet addition to the milk in cheese making indicated that a relationship between calcium and acidic vitamins may exist, it was thought that the addition of calcium chloride to the Cottage cheese making process may also affect its calcium content and, therefore, affect the nicotinic

acid and folic acid contents.

For the manufacture of Cottage cheese with the addition of calcium chloride, the regular short-set method of Cottage cheese making was employed. An analytical grade of anhydrous calcium chloride was dissolved in water and thoroughly mixed with the milk at the time of adding the starter. The Cottage cheese was made from skimmilk to which 0, 0.02 and 0.05% calcium chloride was added, and the cheese was assayed for calcium, niacin and folic acid.

EXPERIMENTAL RESULTS AND DISCUSSION

Vitamin Content of Milk, Whey and Cottage Cheese. Three separate batches of Cottage cheese were manufactured as outlined in the experimental section, and the niacin, B₆, B₁₂ and folic acid content for skimmilk, whey and cheese curd was determined. Then, on the basis of the vitamin content of skimmilk, the vitamin retention was calculated for the cheese curd. The data are presented in Table II.

On the average, the milk contained 71 ug of niacin, 26 ug of B₆, 0.57 ug of B₁₂ and 0.69 ug of folic acid per 100 g of milk. These figures are within the range of values reported in the literature. The average content of these vitamins in whey were 40, 21, 0.53, and 1.10 ug per 100 g, respectively, for niacin, B₆, B₁₂ and folic acid. These data revealed that a considerable portion of the water-soluble B-vitamins were lost in the whey produced during the manufacture of Cottage cheese.

The plain Cottage cheese dry curd contained 257 ug of niacin, 24 ug of B₆, 2.1 ug of B₁₂ and 40.6 ug of folic acid per 100 g of cheese curd. Due to the relatively small number of studies concerning the vitamin concentrations in Cottage cheese reported in the literature, there was no range of values available with which to compare the results obtained in the current work. Considering the few values of vitamins in Cottage cheese reported, the values found here were considerably higher for niacin, B₁₂ and folic acid; whereas the average value for B₆ observed in this study was nearly one-half of that reported in the literature. Factors contributing to these differences in vitamin concentrations may be the vitamin content of milk used in making of the

TABLE II
B-VITAMIN CONTENT OF MILK, WHEY AND COTTAGE CHEESE CURD

Vitamin	Milk		Whey		Cheese curd		Per cent retention
	Range	Ave.	Range	Ave.	Range	Ave.	
-----µg/100 g-----							
Niacin	62-88	71	31-47	40	180-311	257	62.8
B ₆	24-27	26	20-21	21	24-24	24	16.0
B ₁₂	0.56-0.57	0.57	0.50-0.55	0.53	2.0-2.2	2.1	63.7
Folic acid	0.50-0.78	0.69	0.92-1.60	1.10	36.7-45.0	40.6	1018

cheese, the method of vitamin assay used and the treatment of samples prior to the assay.

To determine the amount of vitamin that was retained in the curd from the original milk, the per cent retention of the vitamins was calculated in the cheese curd, which amounted to 62.8% for niacin, 16.0% for B₆, 63.7% for B₁₂ and 1018% for folic acid. These data point out that the retention of folic acid, niacin, and B₁₂ in the curd from the milk was considerably greater than that for B₆. The rather unusually high per cent retention of folic acid in the curd (1018%) and its high concentration in the whey (1.1 ug/100 g) suggested that possibly the starter organisms synthesized vitamins during the setting period of making Cottage cheese.

Biosynthesis of Certain B-vitamins by the Starter. Because of a high retention of vitamins in the curd from the milk, it was felt that the starter possibly synthesized vitamins during the setting period. Therefore, the rate of biosynthesis of the four vitamins during the 16-hr coagulation period used in the long-set method of making Cottage cheese was investigated. Three trials were conducted as outlined in Experimental Methods and vitamin assays were carried out at 4-hr intervals of incubation for niacin, B₆, B₁₂ and folic acid.

As shown in Figure I, the culture synthesized all the four vitamins during the 16-hr setting period. The rates of synthesis of niacin and B₆ were rather slow, but the other two vitamins, B₁₂ and folic acid, were synthesized very rapidly. The niacin content increased from 99 to 126 ug per 100 g, B₆ from 30 to 35 ug per 100 g, B₁₂ from 0.4 to 1.7 ug

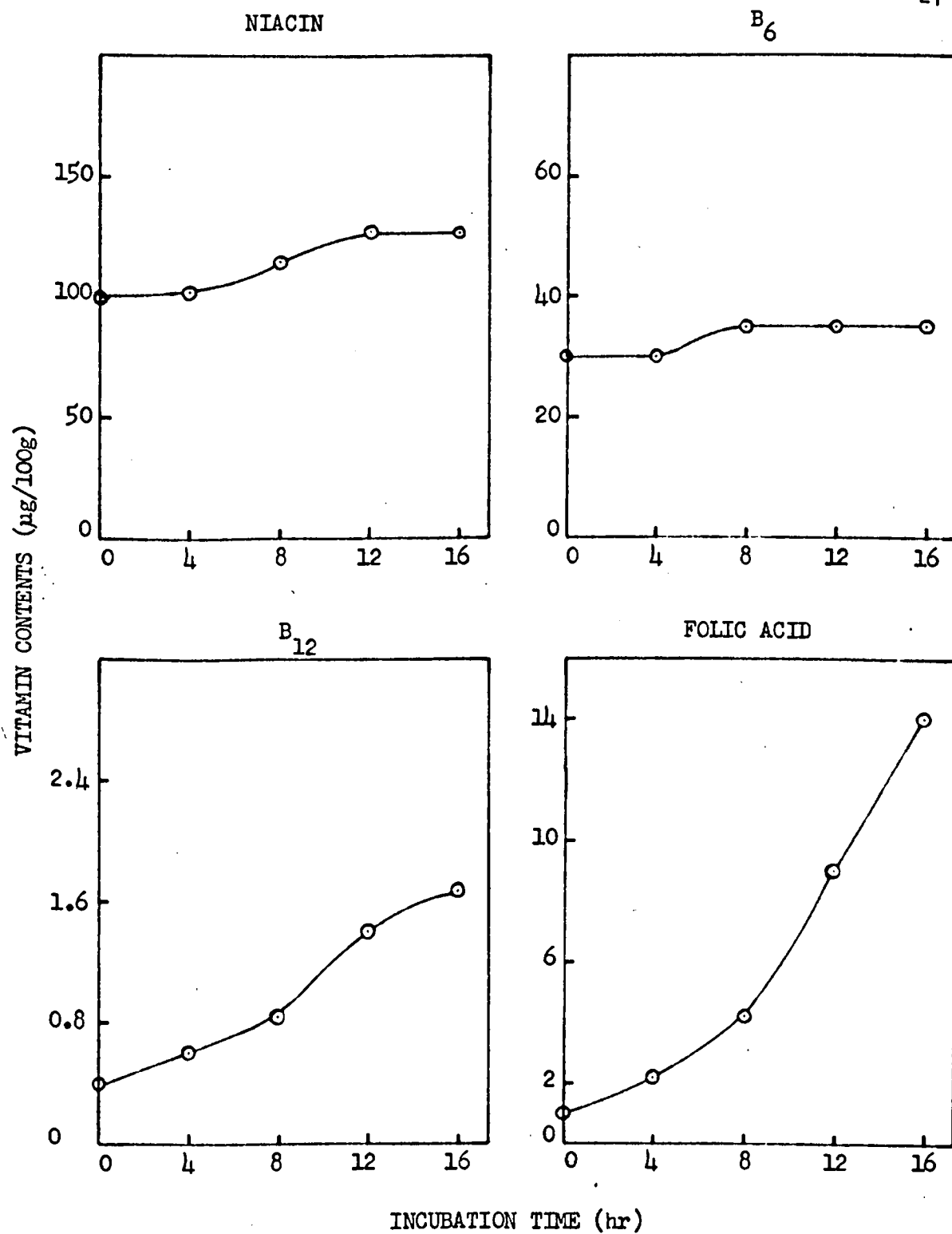


FIGURE I. BIOSYNTHESIS OF CERTAIN B-VITAMINS BY THE COTTAGE CHEESE STARTER.

per 100 g and folic acid from 1.1 to 14 ug per 100 g. The per cent increase of the vitamins during the setting period was 27, 167, 325 and 1270, respectively, for niacin, B₆, B₁₂ and folic acid.

The synthesis of B₆ and folic acid by the lactic acid starter organisms was in agreement with the nutritional studies of Niven (45), Nurmikko (49) and Nambadripad et al. (47) who reported that these vitamins were either synthesized or not utilized by lactic culture organisms.

The synthesis of niacin, as found in this study, was in agreement with the work of Tevilevich (61) who isolated several lactic cultures that synthesized nicotinic acid. However, these results are not in agreement with the results of several workers (1,45,47,54), who found that starter organisms required niacin for growth.

The synthesis of vitamin B₁₂ by our culture did not agree with the results of Laganovskii (33,34) who reported that several lactic cultures did not synthesize vitamin B₁₂. Perhaps certain groups of cultures are capable of synthesizing vitamin B₁₂ and others are not.

In order to take into account the synthesis of the vitamins during the setting period of Cottage cheese manufacture, the retention of vitamins by the curd was recalculated on the basis of the 16-hr coagulum instead of the vitamin content of skimmilk as was normally done. The retention of vitamins, calculated by this method, are presented in Table III, and amounted to 49.3% for niacin, 13.7% for B₆, 14.4% for B₁₂ and 77% for folic acid. These lower retention values, as compared to the previous retention percentages presented in Table II, indicated the

TABLE III
RETENTION OF VITAMINS IN CURD FROM COAGULUM

Vitamin	Milk	16-hr coagulum	Plain Cottage curd	Per cent Retention
-----µg/100 g-----				
Niacin	71.0	126	257	49.3
B ₆	26.0	35.0	24.0	13.7
B ₁₂	0.57	1.7	2.1	14.4
Folic acid	0.69	14.1	40.6	77.0

relative rates of biosynthesis of the four vitamins during the setting period.

Effect of Storage on the Vitamin Content of Cheese. In order to determine the effect of storage upon the vitamin content, the curd (obtained in the preceding phase of work) was creamed, stored in a refrigerator at 50 F, and assayed for niacin, B₆, B₁₂ and folic acid after 1 and 2 weeks of storage. As shown in Table IV, it was observed that the creamed Cottage cheese curd contained less of each of the vitamins than the plain Cottage cheese. This reduction in vitamin concentration was due to the dilution effect of the added cream. The storage of Cottage cheese for two weeks did not cause any significant change in the vitamin content. At the end of the two-week storage period, the samples showed no marked evidence of microbial decomposition, but only a slight stale flavor was detected.

Effect of Rennet Addition to the Milk Upon the Calcium and Vitamin Content of Cheese. Cottage cheese is primarily an acid coagulation process, as compared to the rennet coagulation character of Cheddar cheese. Bosworth (6) reported that in the presence of rennin, the casein molecule was split, forming molecules of soluble paracasein which unite with calcium to form insoluble calcium paracaseinate. Also, McCammon et al. (41) reported that rennet action was responsible for the formation of paracasein which formed insoluble calcium paracaseinate, and resulted in a calcium rich curd. Casein clotted in this way, as calcium paracaseinate on account of the action of rennin, would theoretically

TABLE IV
EFFECT OF STORAGE UPON THE B-VITAMIN CONTENT
OF CREAMED COTTAGE CHEESE

Vitamin	Plain Cottage cheese	Creamed Cottage cheese	Storage	
			1 week	2 weeks
-----µg/100 g-----				
Niacin	257	203	212	220
B ₆	24.0	19.0	19.0	20.0
B ₁₂	2.1	1.9	1.9	1.8
Folic acid	40.6	37.2	37.0	38.0

contain much more calcium than that clotted as insoluble isoelectric casein due to the change in pH of the solution caused by lactic acid. The calcium content of cheese curd might therefore be expected to vary, depending on the method of manufacture.

The methods of manufacture of cheese show that the rennet curd contains more bound calcium than the acid curd. The calcium content of Cheddar cheese was calculated to be about seven times higher than that of Cottage cheese (51). Blunt and Sumner (5) reported that two rennet cheeses, Swiss and Cheddar, contained approximately 14 and 9 times as much calcium as Cottage cheese when calculated in terms of percentage and even more when calculated in proportion to the protein. McCammon et al. (41) reported that Cheddar cheese contained approximately 10 times as much calcium as Cottage cheese and about 20% of the calcium of the milk was retained in Cottage cheese, whereas 80% was retained in Cheddar cheese.

Nicotinic acid and folic acid, being acidic in nature, may exist as salts of other cations in cheese. Therefore, in order to study the possible interrelationship of calcium, niacin and folic acid content of Cottage cheese, studies were made to determine the effect of the addition of different quantities of rennet during the manufacture of cheese upon the calcium, niacin and folic acid content. Four trials of cheese consisting of four batches each were made as outlined in Experimental Methods, and the average calcium and vitamin contents of the curd as a function of the concentration of the rennet added, are presented in Figure II.

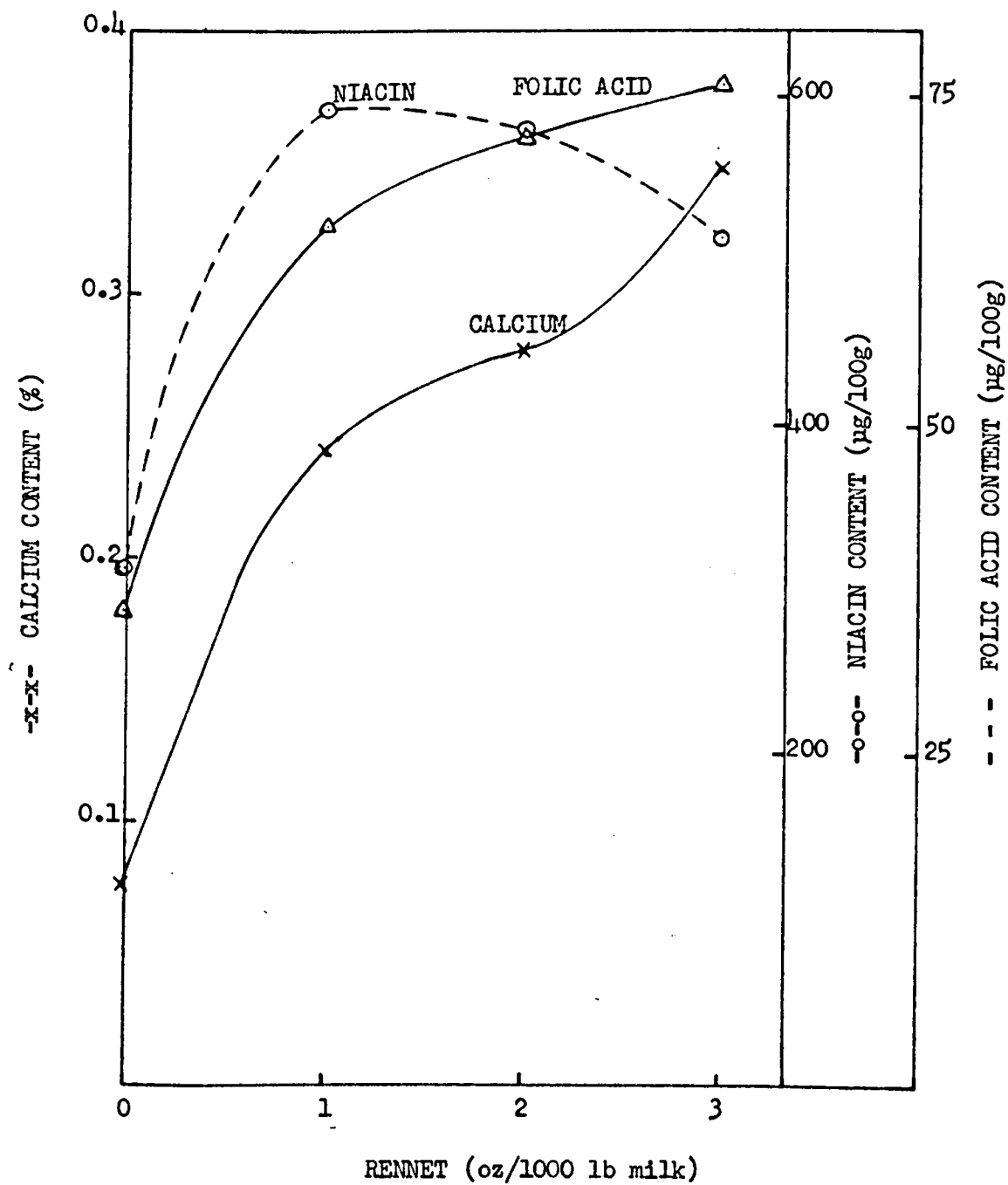


FIGURE II. EFFECT OF THE ADDITION OF RENNET ON THE CALCIUM, NIACIN, AND FOLIC ACID CONTENT OF CHEESE CURD.

With the addition of rennet to the milk used in the manufacture of the cheese curd, there was an increase in the calcium, niacin and folic acid content. There was a direct relationship between the increase in calcium and the folic acid content of cheese curd which indicated that there may exist a direct relationship between the calcium and folic acid content of Cottage cheese. These data indicated that folic acid may exist as a salt of calcium, and by increasing the calcium content, a curd may be produced rich in folic acid.

In the case of niacin content, however, no such direct relationship with the calcium was observed. As pointed out by the graph for niacin, there was nearly a twofold increase in the niacin content with the initial addition of one ounce rennet but further increase of rennet to 2 or 3 oz per 1000 lb resulted in a decrease in the niacin content of cheese. The factors responsible for this decrease remain unknown.

Since the addition of rennet to the Cottage making process resulted in a curd having less moisture content than regular Cottage cheese, it was thought that the calculation of the previous results on a dry-weight basis, instead of the usual moist-weight basis, would give a true picture of the rennet effect on calcium and vitamin content.

The conversion of the moist-weight to dry-weight basis, or mg calcium or ug vitamin per 100 g of dry curd, was calculated as follows:

$$\text{Retention} = \frac{\text{Average calcium or vitamin content of curd}}{\text{Average per cent solids}} \times 100$$

As presented in Figure III, the effect of rennet addition on the calcium and vitamin content, expressed on a dry-weight basis, gave essentially the same general pattern as was observed in Figure II. The

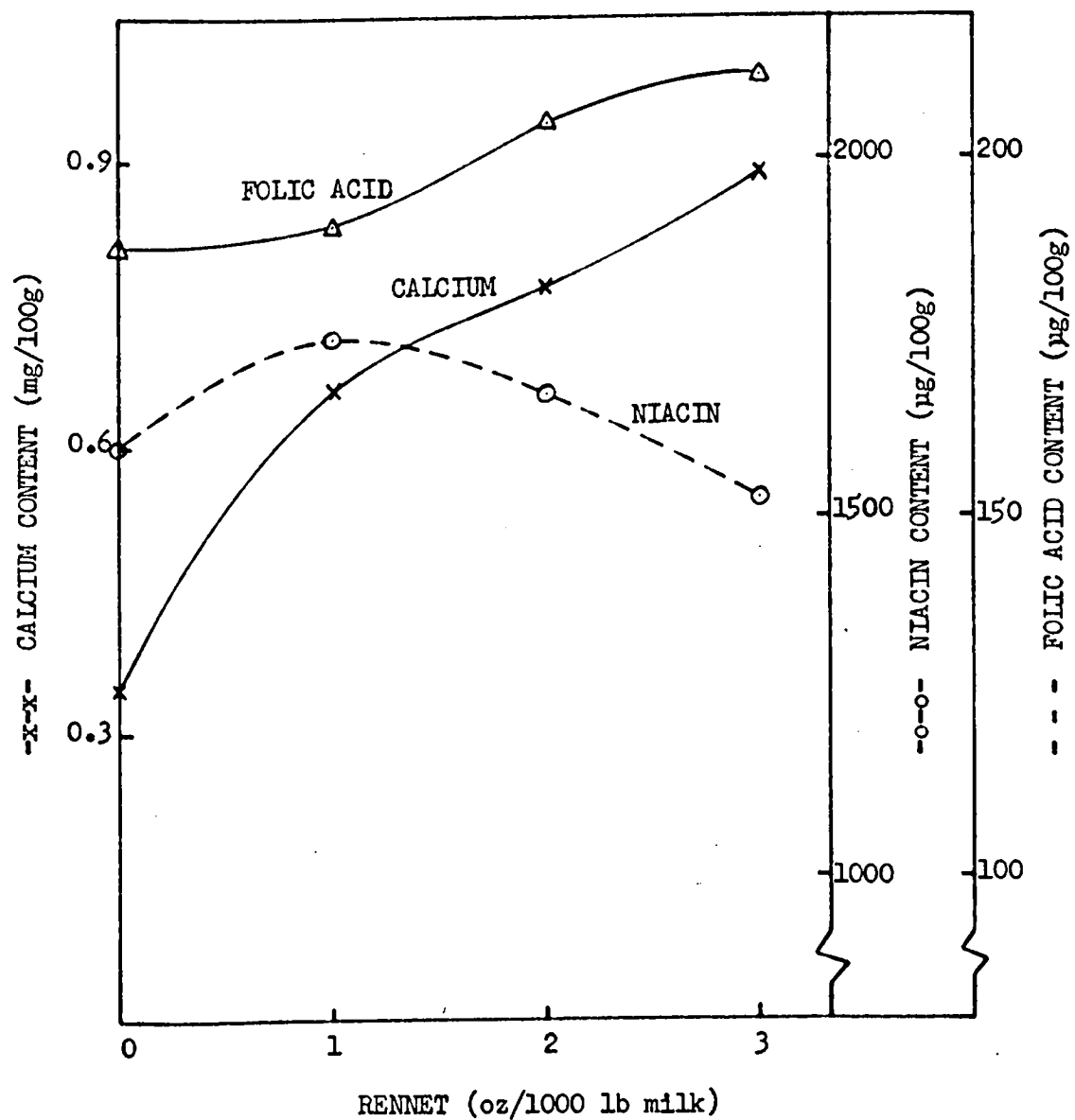


FIGURE III. EFFECT OF THE ADDITION OF RENNET ON THE CALCIUM, NIACIN, AND FOLIC ACID CONTENT OF CHEESE CURD.

most significant difference in patterns of the two sets of curves was found in the region between 0 and 1 oz rennet for calcium and the vitamins. The flattening effect of these curves in Figure III was due to the sudden drop of moisture content between the control and the curd made with the addition of 1 oz rennet. The moisture content of the cheese made by the addition of 1, 2 or 3 oz of rennet per 1000 lb of milk was nearly the same.

Effect of Calcium Chloride Addition to the Milk Upon the Calcium and Vitamin Content of Cheese. Since calcium chloride is often used in the manufacture of Cottage cheese in order to obtain improved curd characteristics, it was felt that there may be a relationship between calcium chloride used in the making of the cheese and its calcium content.

Since earlier work showed a relationship between the calcium and vitamin content of cheese, it was thought that the addition of calcium chloride to the milk used in the manufacture of Cottage cheese may also increase its calcium content and thus affect the vitamins. Therefore, Cottage cheese was made with varying amounts of calcium chloride added to the milk, as described in Experimental Methods, and assayed for calcium, niacin and folic acid.

As can be seen in Table V, the addition of 0.02 and 0.05% calcium chloride to the milk increased the calcium and folic acid content of the curd. These results with folic acid were consistent with those obtained in the preceding phase concerning the rennet addition, again indicating a direct relationship between calcium and folic acid content of Cottage cheese.

TABLE V

EFFECT OF THE ADDITION OF CALCIUM CHLORIDE TO MILK ON THE CALCIUM,
 NIACIN AND FOLIC ACID CONTENT OF COTTAGE CHEESE CURD

CaCl ₂ added	Calcium in curd	Vitamins in curd	
		Niacin	Folic acid
(%)	(%)	-----µg/100 g-----	
0	0	217	31
0.02	0.090	193	42
0.05	0.121	189	47

In direct contrast to the results obtained for folic acid and calcium chloride, the niacin content of Cottage cheese decreased with increasing amounts of calcium chloride.

SUMMARY AND CONCLUSIONS

Vitamin Content of Milk, Whey and Cottage Cheese. Microbiological methods were used to determine the niacin, B₆, B₁₂ and folic acid content of milk, whey and cheese curd. The milk contained on the average 71 ug of niacin, 26 ug of B₆, 0.57 ug of B₁₂ and 0.69 ug of folic acid per 100 g of milk. The average content of these vitamins in the whey was 40, 21, 0.53 and 1.10 ug per 100 g, respectively, indicating that considerable quantities of vitamins were lost in the whey produced during the manufacture of cheese. The Cottage cheese curd contained 257 ug of niacin, 24 ug of B₆, 2.1 ug of B₁₂ and 40.6 ug of folic acid per 100 g of curd. From the vitamin content of the milk used in making the cheese, the per cent retention of the vitamin in the cheese curd was calculated, which amounted to 62.8% for niacin, 16.0% for B₆, 63.7% for B₁₂ and 1018% for folic acid.

Biosynthesis of Certain B-vitamins by the Starter. Unusually high per cent retention of folic acid in the curd from the milk indicated that the starter synthesized vitamins during the setting period. Trials made to determine the rate of biosynthesis of the four vitamins during the 16-hr coagulation period revealed that the starter organisms synthesized niacin and B₆ rather slowly, but the other two vitamins, B₁₂ and folic acid, were synthesized very rapidly. These data revealed that vitamins present in the cheese curd were partly synthesized by the starter during the setting stage of manufacture. On the basis of the vitamin content of the 16-hr coagulum, the per cent vitamin retention in

the curd was recalculated and amounted to 49.3% for niacin, 13.7% for B₆, 14.4% for B₁₂ and 77% for folic acid.

Effect of Storage on the Vitamin Content of Cheese. The storage of creamed Cottage cheese up to two weeks did not cause any significant change in the niacin, B₆, B₁₂ and folic acid content of the cheese.

Effect of the Addition of Rennet to the Milk Upon the Calcium and Vitamin Content of Cheese. Since rennet addition may affect the calcium content of cheese, and the acidic vitamins may be bound to calcium, studies were made to determine the interrelationship of calcium, niacin, and folic acid in Cottage cheese. With the addition of rennet there was an increase in the calcium, niacin, and folic acid content. There was a direct relationship between the increase in the calcium and folic acid content of cheese curd. In the case of niacin, there was a two-fold increase in the niacin content with the initial addition of 1 oz of rennet, but further increases of rennet resulted in a decrease in the niacin content of the curd. These data indicated that there may be some relationship between the calcium and folic acid content of Cottage cheese. Such a relationship between calcium and niacin was not observed.

Effect of the Addition of Calcium Chloride to the Milk Upon the Calcium and Vitamin Content of Cheese. The addition of calcium chloride during the cheese making process may also increase the calcium content of the cheese. Therefore, studies were made to determine the effect of addition of calcium chloride to the milk upon the calcium and vitamin content of cheese. The addition of calcium chloride to the milk

increased the calcium and folic acid content of the curd indicating that there may exist some relationship between the calcium and folic acid content of Cottage cheese. However, the niacin content of Cottage cheese curd decreased with the addition of calcium chloride.

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